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Optimisation of the Post-Harvest Conditions to Produce Chocolate Aroma from Jackfruit Seeds

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ABSTRACT

Jackfruit seeds are an under-utilized waste in many tropical countries. In this work, we demonstrate the potential of roasted jackfruit seeds to develop chocolate aroma. Twenty-seven different roasted jackfruit seed flours were produced from local jackfruit by acidifying or fermenting the seeds prior to drying, and roasting under different time/temperature combinations. The chocolate aroma of groups of four flours were ranked by a sensory panel (n=162) and response surface methodology was used to identify optimum conditions. The results indicated a significant and positive influence of fermentation and acidification on the production of chocolate aroma. SPME/GC-MS of the flours showed that important aroma compounds such as 2,3-diethyl-5-methylpyrazine and 2-phenylethyl acetate were substantially higher in the fermented product, and that the more severe roasting conditions produced 2-3 times more 2,3-diethyl-5-methylpyrazine, but less 3-methylbutanal. Moisture, a_w , pH, luminosity and color were also monitored to ensure that these properties were similar to cocoa powder or cocoa substitutes.

Keywords: jackfruit seeds, chocolate aroma, waste utilization, sensory analysis, SPME/GC-MS

INTRODUCTION

Jackfruit (*Artocarpus heterophyllus* Lam.) is a large tropical fruit which is abundant in South America, Asia, Africa and Australia. It is a fleshy compound fruit (syncarp) belonging to the Moraceae family and takes 3-6 months to reach ripeness. The fruit weight ranges from 2 to 36 kg and its seeds account for around 15-18 % of the total weight of the fruit.^{1,2} Generally the seeds are boiled, steamed and roasted before eating, providing a cheap source of fiber, protein and minerals. In many countries, including Brazil, jackfruit seeds are an under-utilized waste stream.

There are several publications reporting the use of waste jackfruit seeds to produce starch,³⁻⁶ but there is little reported in the literature on their potential to generate flavor. For the first time we found that after roasting, jackfruit seeds imparted an aroma similar to chocolate. Chocolate aroma has been well-characterized^{7,8} and a number of different aroma compounds have been found to contribute to the complex and characteristic aroma of chocolate. The most odor-active compounds in milk chocolate identified by Schnermann and Schieberle⁷ include 3-methylbutanal, phenylacetaldehyde and 2,3-diethyl-5-methylpyrazine and, a few more in roasted cocoa beans.⁹ Some pyrazines have been shown to contribute significantly to the unique flavor of roast and toast foods⁹ and are used to determine the quantity and quality of cocoa flavor.¹⁰ They impart chocolate, cocoa, hazelnut, roasted, coffee, earth and green aromas.^{11,12} As with cocoa, the post-harvest pre-treatments and roasting of the jackfruit seeds are likely to influence the formation of these compounds and the quality of the aroma.

All three stages of the process (fermentation, drying and roasting) can have an influence on the final pyrazine concentration. During fermentation, enzymatic and microbial processes induce physical and chemical changes in seeds which result in

browning reactions.¹³ Some volatile compounds are formed at this stage, as well as free amino acids and sugars which are substrates for the subsequent flavor-forming reactions¹⁴ which take place during roasting. The influence of fermentation parameters on the aroma of roasted cocoa beans is well understood and has been reviewed recently.¹⁵ Kirchhoff et al.¹⁵ demonstrated that chocolate aroma was correlated to proteolysis and the subsequent accumulation of free amino acids. The proteolytic enzymes such as endopeptidases and proteases are highly sensitive to pH, so pH control is important during cocoa fermentation to regulate the activity of different enzymes. These products of fermentation (amino acids and reducing sugars) are the precursors of pyrazines which are formed during roasting in the Maillard reaction.^{16–19}

Cocoa (*Theobroma cacao*) is a culture which is highly sensitive to changes in climate, is susceptible to many typical diseases and local farmers struggle to compete with international cocoa suppliers.^{20,21} Global cocoa production is around 3.7 million tons and this is not expected to grow significantly in the next 10 years,²² however demand by 2020 is estimated to be 4.5 million tons.²¹ In this context, new sources of chocolate aroma and flavor are important to meet the increase in demand and provide alternative revenue streams for local farmers and communities in Brazil.

The aim of this work is optimize the production of chocolate aroma from jackfruit seeds by treating them under conditions similar to those used in the cocoa process. Seeds will be acidified or fermented prior to drying, and roasted under different time/temperature combinations. Sensory ranking tests will be used to assess the chocolate aroma and key aroma compounds will be analysed by SPME/GC-MS.

MATERIALS AND METHODS

Chemicals. Standards of 3-methylbutanal, phenylacetaldehyde, 2-phenylethyl acetate, 2-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2,3-dimethylpyrazine, 2,3,5-trimethylpyrazine, 2,3,5,6-tetramethylpyrazine, 2,3-diethyl-5-methylpyrazine, 1,2-dichlorobenzene and the alkane standards C₆–C₂₅ were purchased from Sigma Aldrich Química, São Paulo, Brasil.

Jackfruit. Twenty five jackfruit of the hard pulp varieties were manually collected from one single tree, between October 2013 and January 2014, in the countryside of São Paulo, Brazil, selecting fruit of similar size (5 ± 1 kg) and ripeness, as indicated by the yellow color of the shell. Jackfruits were cleaned manually in running water, the seeds removed and the pulp discarded. These seeds were subjected to one of three different treatments prior to roasting, producing either dried jackfruit seeds (DJS), acidified jackfruit seeds (AJS) or fermented jackfruit seeds (FJS). For each treatment, the seeds from 7-9 jackfruit were pooled, and treated and dried in four \times 1.5 kg batches (3 kg batch for FJS) as described below. The dried beans (50 g from each of the four batches) were roasted in 200 g portions. In total, 11 bags of roasted flour (200 g) were prepared for each of the three treatments.

Seed processing. For the dried jackfruit seed (DJS), the seeds were dried in an oven at 60 °C with air circulation. After 24 h, the spermoderms were manually removed, and the seeds remained for a further 24 h in the same oven at the same temperature.

For the acidified jackfruit seeds (AJS), treatment was carried out at ambient temperature (25 ± 3 °C). For each batch, the seeds (1.5 kg) were placed in polyethylene trays (28 x 42 x 7.5 cm) with 1% w/w acetic acid in potable water (3 kg). After five days the solution was removed and the seeds were dried using the same method as for DJS (2 \times 24 h).

For the fermented jackfruit seeds (FJS), simulating what is done with cocoa, seeds (3 kg) were placed in polyethylene boxes to ferment with added jackfruit pulp (1.5 kg), perianth (0.52 kg), and banana leaves (0.1 kg) as a source of yeast. For the first 6-7 days of fermentation, the boxes were closed to promote anaerobic fermentation but, for the remaining 7-8 days, the boxes were opened and the fermenting mass was rolled daily to promote oxidation. The seeds were removed and dried using the same method as for DJS (2 × 24 h). These processes are summarized in Figure 1A. The yield from each treatment was expressed as in equation 1.

$$\text{Yield (\%)} = (\text{weight of flour after drying}) \times 100 / \text{weight of raw jackfruit seeds} \quad \text{Eqn1}$$

During acidification and fermentation, the ambient temperature and the temperature of the fermenting mass were measured every 24 h²³ (AOAC Methods 13.010; 32.010; 32.016 and 32.017).. For AJS, the pH of an aliquot of liquid extracted from the mass in the polyethylene boxes was measured directly. For the FJS mass, 10 g of the fermenting mass was added to 100 mL of distilled water. In both cases, the pH was measured using a pH meter with a glass electrode standardized at the experiment temperatures over the range from 7.0 to 4.0. For FJS, total titratable acidity (AOAC 945.08)²⁴ was measured using 5 g fermenting mass, diluted 10 times and filtered. Proximate analysis was carried out according to Horwitz et al.²³

Roasting and Grinding. For each treatment, 11 batches of seeds (200 g) were roasted in a rotary electric oven (Probat[®] laboratory sample roaster, Emmerich am Rhein, Germany) with digital temperature control, using conditions defined by the response surface methodology. A central composite design was used for each treatment (Figure 1B). Two factors (roasting time and temperature) were each tested at five levels, with three repetitions of the central point totaling 11 samples. However, preliminary experiments showed it was necessary to select different roasting

conditions for each treatment to avoid burning of the FJS yet achieve significant roasting in the AJS. The temperature ranged from 150 to 201°C ± 0.1°C and the roasting time from 33 to 47 min. The roasted seeds were then milled in a hammer mill to produce a “flour”. There was no heating of the sample during milling, minimizing loss of volatile compounds at this stage. Flours were packed under vacuum and stored without light at 8±1 °C.

Analysis of Flours. Water activity was determined from the temperature of the dew point (Aqualab®), moisture was determined by a standard gravimetric method, and color was measured instrumentally using a Minolta® colorimeter, with illuminant C, previously calibrated with a white surface (Y = 93.7, x = 0.3135 and y = 0.3195) based on the CIE-lab L,* a,* b,* scale. The pH was determined in triplicate using 2 g of flour added to distilled water (20 mL). The quality of the chocolate aroma was based on a sensory comparison and the relative concentration of selected aroma compounds was measured by GC-MS. The proximate composition was only carried out on flours with the highest sensory rankings.

Sensory analysis. All sensory evaluations were approved by the Ethics Committee of Human Research of the ESALQ/USP (COET/077/131).

Preliminary sensory tests. Preliminary tests were carried out to determine the optimum temperature and time for sample exposure prior to the panelists receiving the sample for assessment. In this preliminary assay using AJS flour, the samples were placed in a water bath for five different combinations of time (30, 60, 120 s) and temperature (25, 36.5, 48 °C) prior to sniffing by a small panel comprising 21 untrained members aged 18-40 years (76% women). Each panelist was asked to rank groups of three samples in increasing order according to the intensity of the chocolate aroma

(Table 1). There was no significant difference between the conditions used to equilibrate the samples so the conditions were standardized at 40 °C for 120 s.

Sensorial ranking test. Ranking tests were used to determine the relative intensity of chocolate aroma in 11 samples for each treatment (DJS, AJS and FJS) using incomplete blocks (Figure 2). Each sample (3 g) was placed in an amber vial coded with a random three-digit number and, prior to sensory evaluation, the vial was heated for 120 s in a water bath at 40 °C, these conditions having been selected from the preliminary tests. Panelists received simultaneously four coded samples to rank in increasing order of intensity of chocolate aroma, from least (=1) to most (=4). Total ranking scores were used, thus the higher score representing the greater chocolate aroma. The data obtained from the panelists were collected and analyzed using Compusense five.[®] At the end of the session, the panelists were asked to describe different aromas they identified in each of the samples using their own free choice of descriptors.

Sensory experimental design. Untrained panelists (162) aged 18-54 years (60% women) were randomly divided into three equal groups of 54 – one group for each treatment (DJS, AJS and FJS). In order to reduce the number of comparisons to be assessed by the panel, a balanced, incomplete block, design of experiment was used²⁵ to construct a second order model based on the 11 samples in the central composite design.²⁵ However, to minimize panelist fatigue, the central point was represented by a blend of the three central points (reducing the number of samples to 9) and the three central points were assessed by the same panelists in a second sensory session.

In the first sensory session panelists received four samples in a balanced incomplete block (Figure 2). Each sample block consisted of 18 comparisons (9 samples each appearing 8 times). The sample block was repeated three times (for 54 panelists)

and the parameters, as defined by Cochran and Cox²⁴, were T=9; k=4; r=8; B=18; L=3; E=84; Z=3.

In the second sensory session, the same panelists received three samples of the central point (0, 0) (Figure 2). These samples were delivered at the same time, in a randomized and balanced complete block²⁵. Total ranking scores from the second session were transformed to be comparable to the first sensory session. Thus it was possible to assess the variation between the central points and validate the response surface for intensity of chocolate aroma.

Volatile analysis. Jackfruit flour (DJS, AJS and FJS) (3 g) was placed in a 20 mL SPME vial with 1 μ L of 1,2-dichlorobenzene in methanol (130.6 μ g/mL) and vortexed for 2 min. After equilibration at 45 °C for 15 min, the triple phase fiber (65 μ m PDMS/DVB/Carboxen from Supelco) was exposed (1cm) to the headspace above the sample for 55 min under magnetic agitation (635 rpm). These conditions had previously been optimized using surface response methodology.

The volatile compounds extracted by the fiber were analyzed by GC-MS using a Shimadzu® QP2010 GC-MS equipped with a RTX5MS column (30 m, 0.25 mm i.d., 0.25 μ m film thickness). Volatile compounds were desorbed for 1 min during a splitless injection at 200 °C. During desorption, the oven was maintained at 40 °C for a further 8 min, and then the temperature was raised at 4 °C/min to 200 °C, and then 10 °C/min to 280 °C totaling 56 min. MS was carried out using 70 eV electron impact, and *m/z* were monitored in the range 40 to 500, in scan mode. Helium was the carrier gas and the flow rate was 1 mL/min in constant flow. A series of n-alkanes C₆–C₂₀ was analyzed under the same conditions to obtain linear retention indices (LRIs) for comparison with authentic samples. All volatile compounds listed were identified by comparison of their

mass spectrum and LRI with that of an authentic standard run under similar conditions. Each sample was analyzed three times.

Peak areas for 3-methylbutanal, phenylacetaldehyde and 2-phenylethyl acetate were measured using the total ion chromatogram. For the following compounds, the peak area was approximated using the area of a characteristic m/z which was multiplied by a factor calculated from the spectrum obtained from the authentic standard: 2-methylpyrazine m/z 94, factor 3; 2,5/6-dimethylpyrazine (coelute) m/z 108, factor 2.5; 2,3-dimethylpyrazine m/z 67, factor 4; trimethylpyrazine m/z 81, factor 14; tetramethylpyrazine m/z 54, factor 5; 2,3-diethyl-5-methyl-pyrazine m/z 121, factor 20. The approximate relative concentration of each compound was obtained by comparing the peak area against that of the internal standard (1,2-dichlorobenzene), using 1 as a response factor.

Statistical analysis and response surface methodology. The central composite design, Statistics® (2014), was selected for use. The two key responses were intensity of chocolate aroma, 3-methylbutanal and 2,3-diethyl-5-methylpyrazine concentration, although water activity, moisture, pH and color were also monitored. The two independent variables of the design, roasting time and temperature, were coded as x , and y , respectively. Equation 2 shows the quadratic polynomial model that was fitted to each response, where b_0 , b_1 , b_2 , b_{11} , b_{12} and b_{22} are the regression coefficients; x and y are the values of the independent variables for roasting time (min) and temperature (°C) respectively.

$$z = b_0 + b_1x + b_2y + b_{11}x^2 + b_{22}y^2 + b_{12}xy \quad \text{Eqn 2}$$

The analysis of variance (ANOVA) tables were generated and regression coefficients of individual linear, quadratic and interaction terms were determined by using design expert software (Statistics®). The significances ($p \leq 0.05$) of all terms in the

polynomial model were judged statistically by computing the F value. XLStat was used to carryout 2-way ANOVA on the volatile data and calculate Fisher's least significant difference at $p=0.05$.

RESULTS AND DISCUSION

Jackfruit seed processing. In cocoa beans, control of the fermentation process is required because unfermented beans develop little chocolate flavor, and excessive fermentation may also result in unwanted flavors when roasted.²⁶ Generally for cocoa, fermentation lasts between 5 to 8 days, and the end point is determined by experience²⁷ based on reducing acid notes and maximizing chocolate flavor in the final roasted product.²⁸⁻³⁰

In this study, the fermentation process of 12 days was necessary, maybe because jackfruit seeds are bigger in comparison to cocoa beans and there is more substrate to ferment. During the fermentation it is important to kill the embryo at the beginning to ensure the success of the fermentation process and the formation of flavor compounds. Jinap and Dimick³¹ and Heemskerk et al.³² reported that a pH close to 4 would destroy the embryo; in jackfruit we found this value around day 3-4 of fermentation (Figure 3A) whereas the acidification process started at pH 3 and fluctuated between pH 3 and pH 4 (Figure 3B). In cocoa, samples are considered well fermented at $pH > 4$ although this varies with variety.²⁸ In practice, an increase in pH of the seeds has been shown to improve chocolate flavor during fermentation and alkalization²⁹⁻³³ reported that pH values lower than 4.5 in the seeds decreased the aromatic potential of the cocoa beans. So there is a balance between achieving a pH which is low enough to kill the embryo but high enough to form aroma compounds.

Although titratable acidity in FJS was very variable, the overall trend was for an increase as the pH dropped (Figure 3A and 3C). Rodriguez-Campos et al.³⁴ reported

similar results during cocoa fermentation with a correlation coefficient of -0.91 between pH and titratable acidity, and -0.86 for the correlation of the concentration of acetic and lactic acid with pH.

Acetic and lactic acid are present in the first and second stages of cocoa fermentation, when anaerobic yeasts and lactic acid bacteria are present, respectively. Towards the end of fermentation, when aeration increases, the acetic acid bacteria become more significant. They are responsible for converting alcohol to acetic acid, and since this reaction is exothermic (Figure 3D), it is likely that they are also responsible for the increase in temperature of the fermenting jackfruit mass.³² At the end of the jackfruit fermentation period (day 12), the temperature of the mass had risen from ambient to values near to 40 °C, similar to the rise during fermentation of cocoa beans although, in cocoa, temperatures can reach 45 °C.²⁸ Figure 3 shows the pH, titratable acidity and temperature profile for FJS and pH for AJS.

For such a natural and variable process, these figures show that, with the exception of titratable acidity, these processes are fairly reproducible. In addition, it shows that jackfruit seeds can be fermented and dried under similar, albeit slightly longer, conditions to those applied to cocoa beans, resulting in a similar drop in pH which in cocoa results in the formation of aroma precursors.

Yield, pH, water activity (a_w), moisture, luminosity (L^*) and chroma (c^*) of jackfruit seed flours. In terms of total mass, the yields of flour obtained from DJS, AJS and FJS were 48%, 45% and 40% respectively.

The pH of the roasted jackfruit seed flours were highest (pH > 5) in the flours which had been roasted at the highest temperature (independent of seeds processing) and the lowest pH (< 4.9) was found in general in the FJS flour (Table 2). These pH values are similar to those reported in traditionally fermented and roasted cocoa (4.75

to 5.19).^{28,33-36} In other cocoa substitutes, Yousif and Alghzawi³⁷ found roasted carob powder to be pH 4.81 and Queiroz and Garcia³⁸ reported the pH of roasted cupuaçu flour as 4.77 - both similar to fermented and roasted jackfruit seeds (Table 2).

The pH of the flours can be fitted to a 3-dimensional surface as a function of time and temperature by using a combination of linear and quadratic terms, as well as an interaction term, to construct a polynomial equation. The correlation coefficient (r^2) indicates how well the data fit the model, and the p-value associated with each coefficient in the equation indicates the certainty with which this term influences the response (Table S1). The correlation coefficient is good ($r^2 > 0.7$) so it is possible to model and predict the pH of the flour from AJS and FJS as a function of time (x) and temperature (y) using equations 3 and 4 respectively. In FJS flour (Eqn 4), there was a linear and quadratic relationship with temperature ($p = 0.006$ and 0.03 respectively) and a linear correlation with time ($p = 0.04$). For AJS flour we found significant linear effects with temperature ($p = 0.01$). However, for DJS flour, the final pH was relatively insensitive to changes in the roasting conditions and the model cannot be used predictively ($r^2=0.6$). The pH was on average higher in flours from DJS compared to AJS and FJS.

$$pH_{AJS}=37.455-0.42557x-0.27377y+0.001036x^2+0.000566y^2+0.0019444xy \quad (r^2=0.81)$$

Eqn 3

$$pH_{FJS}=19.94+0.0899x+0.2223y-0.00159x^2+0.0006789y^2+0.0002987xy \quad (r^2=0.93) \quad \text{Eqn 4}$$

Generally the moisture was associated with water activity (a_w) in flours, and both tended to decrease as roasting conditions became more severe (Table 2). In FJS flours, the highest roast temperature (180 °C) for 40 min (0, 1.41) produced the lowest a_w and the lowest moisture was obtained at 186-192 °C for a 35-40 min roast. In this study we found 2.3% moisture in flour from FJS at (0, 1.41) which was high compared to DJS and

298 AJS flours roasted under similar conditions. By comparison, Yousif and Alghzawi³¹
 299 found 9.0 and 2.5% moisture for roast carob powder (150 °C for 60 min) and cocoa
 300 powder respectively, and Queiroz and Garcia³⁸ showed 3.0% moisture in roasted
 301 cupuaçu powder. The a_w described for both these substitutes was around 0.4. Thus
 302 flours of jackfruit seeds have similar or lower moisture and a_w in comparison to cocoa
 303 and other substitutes, which is important to restrict microbial growth in the flours and
 304 for application in other products. The surface response design allows use of equations
 305 5, 6 and 7 (x = time and y = temperature) to predict the moisture in the flour of DJS, AJS
 306 and FJS ($r^2 > 0.7$); in all equations we could observe the significant linear effect of both
 307 roasting time and temperature in determining final moisture content (Table S1).

308 $\text{Moisture}_{\text{DJS}} = 65.71 - 0.2845x - 0.6427y - 0.003497x^2 + 0.001512y^2 - 0.000383xy$ ($r^2=0.97$;
 309 linear temperature effect $p=0.001$) Eqn 5.

310 $\text{Moisture}_{\text{AJS}} = 39.15 - 0.349499x - 0.26448y - 0.0067x^2 + 0.0006943y^2 - 0.0012498xy$ ($r^2=0.92$;
 311 linear temperature effect $p=0.010$) Eqn 6.

312 $\text{Moisture}_{\text{FJS}} = 105.447 - 1.6209x - 0.7213y + 0.000926x^2 + 0.001246y^2 - 0.0050106xy$ ($r^2=0.90$;
 313 linear temperature effect $p=0.020$) Eqn 7.

314 In contrast, a_w , where there was much greater variability in the responses, can
 315 only be predicted in DJS flour and only the linear term in temperature was significant
 316 (equation 8), and negative, showing that as the temperature increased, the a_w
 317 decreased.

318 $a_{w\text{DJS}} = -4.454 + 0.1165x + 0.03237y - 0.0011974x^2 - 0.000087113y^2 - 0.000127xy$
 319 ($r^2=0.75$; linear temperature effect $p=0.04$) Eqn 8.

320 Color in food is important because appearance can contribute to recognition,
 321 perception and enjoyment of the food. For substitutes, it is necessary to match the
 322 original product as closely as possible. In cocoa powder the luminosity (L^*) is low (near

to black and brown), similar to the jackfruit flour which was produced from the high temperature roasts. L^* tended to be lower (darker) in FJS compared to AJS flour. For chroma, the results were the reverse with high intensity color (larger chroma value) in the higher roasts, and the FJS flours having the least intense color, although there were few significant differences between roasting treatments. Luminosity results for fermented jackfruit seeds were similar to values in roasted cupuaçu. Cohen and Jackix,³⁹ reported L^* of 42 in cupuaçu liquor compared to values of 50-70 found in the jackfruit. Sacchetti et al.⁴⁰ found $L^* = 21$ for roast cocoa beans (145 °C to 30 min) and Sengül et al.⁴¹ found $L^*=19$. Only Gu et al.¹² had slightly higher luminosity ($L^*= 41$) for roast cocoa (160 °C for 30 min). Therefore depending on the kind of product developed using jackfruit seed flour, it may be necessary to modify the color with other ingredients. It is possible to predict the luminosity and chroma of DJS flour using equations 9 and 10 (x = time and y = temperature, $r^2 > 0.7$). In both equations we observed a significant negative linear effect ($p \leq 0.05$) of roast temperature (i.e. as temperature increased, L^* decreased and the product became darker), and, for DJS, roast time was also significant. For acidified and fermented flours we found no significant effect of roasting conditions (r^2 was 0.60 and 0.51 for chroma; and for luminosity 0.44 and 0.52 for AJS and FJS respectively).

$L^*_{DJS} = 70.645 - 0.9291x - 0.2478y + 0.005709x^2 + 0.0000373y^2 + 0.0060888xy$ ($r^2=0.94$; linear temperature effect $p=0.007$; linear time effect $p=0.0002$) Eqn 9

$Chroma_{DJS} = 19.39 + 0.27954x - 0.47968y - 0.008682x^2 - 0.00159939y^2 + 0.002884xy$ ($r^2=0.88$; linear temperature effect $p=0.03$) Eqn 10

Proximate composition of jackfruit seed flours. The proximate analysis was only carried out on the three best roast conditions determined by sensory score (Table 3). For DJS flours, where there was no significant difference between the samples in

terms of sensory score, a sample with high pyrazine content and a high sensory score was selected. The different treatments produced different proximate composition. The moisture was smallest in AJS flours, maybe because during five days in acetic acid solution the seed had dehydrated. AJS and DJS were similar in proximate content. In FJS, the fermentation process results in the breakdown of carbohydrates and the release of CO₂. This is reflected in the proximate analysis where the remainder of the material is assumed to be carbohydrate. This is significantly lower in FJS (53%) compared to DJS (65%) and AJS (73%) respectively. The indirect consequence of this is a small increase in the % contribution from the other analytes.

Moisture, a_w , pH and color of the roasted jackfruit flours tended to vary with the time and temperature of the roasting conditions. However, the pH and moisture of the milled flours were similar to those of cocoa powder, and although the color was a bit pale (high L*), these flours have similar properties to cocoa, carob and cupuaçu powders, and could be used in similar products.

Sensory assessment of chocolate aroma of jackfruit flours. The response surfaces for the sensory ranking tests are shown in Figure 4 A-C and the data are shown in Table 4. The correlation coefficients for the 3D surface models for all three processes (dry, acidified and fermented) were ≥ 0.7 . For DJS flours (Figure 4A), there was no significant difference between samples ($p \leq 0.05$) in the perception of sensory chocolate aroma, although the model showed a linear effect with temperature ($p \leq 0.03$) suggesting that the higher temperature may increase slightly the chocolate aroma. For AJS flours, roasting at the temperature of the central point (180 °C) generated the greatest sensory perception of chocolate aroma (Table 4). The model showed a clear quadratic effect with temperature ($p \leq 0.02$) shown in Figure 4B, which is also represented by a significant coefficient for y^2 ($r^2 = 0.86$) in the corresponding

equation, indicating a decrease in chocolate aroma as the roasting conditions became more severe (and possibly over-cooked from a sensory perspective). However, the most sensory chocolate aroma was found in FJS flours. The sensory rankings of chocolate aroma (SCA) were 72 for FJS (40 min to 150 °C) compared to 70 for AJS (40 min to 180°C) and the average of DJS was 60. Clearly, a fermentation or acidification process is necessary to produce chocolate aroma using jackfruit seeds, and it is possible to select the best roasting conditions for each treatment to optimize the sensory perception of chocolate aroma.

A range of descriptive terms were collected for the flours (Figure 5). All treatments were described with chocolate and coffee terms. In addition, sweet aroma attributes were used to describe DJS flours (honey, milk, etc.) suggesting a relatively mild processing treatment. Unfermented cocoa is very bitter and astringent with little apparent chocolate flavor^{27,35} whereas the unfermented jackfruit flour (from DJS) still had some chocolate aroma. For AJS flour, sweet aromas such as vanilla were similar to DJS flour, but other descriptors were used (e.g. earthy, rancid, acid, silage, fermented, green, etc.) which suggest that the chemical acidification process (rather than the natural fermentation process) may produce less desirable attributes which are not directly associated with food. However, FJS flour was described with fruity qualities (orange, passion fruit, cherry, jackfruit and guava). These aromas are likely to be related to fruity aldehydes, alcohols and esters which are products of the fermentation process. FJS flour was also described with caramel, soya, hazelnut and roast attributes suggesting a greater contribution from the Maillard reaction.

Overall, the sensory evaluation confirmed that a chocolate aroma can be generated from roasted jackfruit seeds, and demonstrated that it can be influenced by both the seed processing and the roasting conditions. The optimum chocolate aroma

score was obtained under moderate roasting conditions when the seeds had been fermented in a process similar to that used for fermenting cocoa beans, or acidified with acetic acid prior to roasting. However, the latter was described by the panel with additional less desirable terms. The best conditions were not necessarily obtained from the most severe roasting conditions and, for AFS flour, there was a very clear optimum, after which there was a decrease in chocolate aroma as the roasting conditions became more severe.

Volatile aroma compounds in jackfruit seed flours. Selection of aroma compounds was based on a survey of the literature (1997-2017), considering only those papers where the odor-active compounds in chocolate or other cocoa products had been established using GC-Olfactometry.^{7,8,25-27} From each paper, the 15-20 most important aroma compounds for chocolate or cocoa aroma were identified and collated, based on either their flavor dilution factors (FD), odor activity values (OAV) or frequency of detection. The results of the survey are shown in Table S2. Chocolate aroma is a complex mixture of 30-50 odor-active compounds, none of which imparts a recognisable chocolate note. Some are present at very low concentrations (e.g. 2-acetyl-1-pyrroline, 4-hydroxy-2,5-dimethyl-3(2H)-furanone), often below the detection threshold when using SPME. Others, although contributory, are reminiscent of aromas very different to that of chocolate (e.g. 3-methylbutanoic acid, 2-methyl-3-methyldithio)furan and 1-octen-3-one which impart cheesy, meaty and mushroom aromas respectively). In choosing just a few key compounds to monitor, our criteria were based on selecting those which had previously been identified as having high FD factors and high OAVs in chocolate or cocoa products, those which were relatively abundant, and those which had a relevant aroma. On this basis we selected 3-methylbutanal, one of the most abundant compounds and also one which at the

appropriate dilution can be described as cocoa and malty. Phenylacetaldehyde and 2-phenylethyl acetate were selected as compounds which contribute the floral character to chocolate. 2,3-Diethyl-5-methylpyrazine and trimethylpyrazine were selected as compounds which contribute the nutty earthy character. The approximate relative contributions of these, plus four other pyrazines, are shown in Table 5.

The most obvious difference is the fact that in the FJS flours, all selected volatiles, except 3-methylbutanal, were present at significantly higher concentrations compared to the respective AJS and DJS flours, particularly the pyrazines, and 2-phenylethyl acetate which was 50 times higher across all conditions. Since these compounds are amongst those which have been shown most frequently to be associated with chocolate aroma (Table S2), and have also been shown to be amongst the most odor-active, it is highly likely that these compounds are responsible for the high sensory scores for chocolate aroma in FJS.

Table 5 shows the significant differences within each pre-treatment (AJS, FJS or DJS). 2-Way ANOVA showed that for most compounds, under all treatments, there was a highly significant difference between flours prepared at different temperatures. In some cases, the roasting time was also significant, and the interaction between the two was significant in some cases.

It is interesting, however, that the key aroma compounds behaved quite differently with roasting time and temperature. With all three pre-treatments (AFS, FJS and DJS), 3-methylbutanal and phenylacetaldehyde showed a tendency to decrease as the more severe roasting conditions were employed. 3-Methylbutanal is both highly volatile and highly reactive: for example it readily undergoes aldol condensations with other aldehydes. Either or both of these may explain the decrease in concentration as the severity of the roasting process increased. This decrease in 3-methylbutanal may

also contribute to the decrease in chocolate aroma which was observed particularly in
AJS and also in FJS as the roasting conditions became more extreme.

The trends for 2-phenylethyl acetate were not clear or consistent, and within
each pre-treatment group, the differences due to different time-temperature
combinations were small or non-significant.

2,3-Diethyl-5-methylpyrazine, the most odor-active of the pyrazines identified in
most chocolate and cocoa products, showed a tendency to increase with increasing
severity of the roasting conditions, as is often the case for pyrazines. However, for
trimethylpyrazine, another important compound in chocolate aroma, the trends were
less clear, and in FJS it (and tetramethylpyrazine) tended to decrease with more severe
conditions, although both tended to increase slightly in AJS and DJS. The
dimethylpyrazines also tended to increase with increased roasting conditions in AJS
and DJS, but did not vary much in FJS.

In AJS and DJS, as the roasting conditions became more severe, the 3-
methylbutanal decreased whereas the 2,3-diethyl-5-methylpyrazine increased. Both
being important for chocolate aroma, this is consistent with the sensory data which
showed an optimum sensory chocolate aroma under moderate roasting conditions for
AJS and DJS. In addition, the more severe conditions might also promote the formation
of other pyrazines which at higher concentration would impart more roasted and
burnt notes, as described in some DJS and FJS samples.

In FJS, most of the compounds were not sensitive to changes in roasting
conditions, although 3-methylbutanal, phenylacetaldehyde and trimethylpyrazine
tended to decrease. This is consistent with the sensory perception of chocolate aroma
in FJS which showed a tendency to decrease as the roasting temperature increased.

Response surface methodology.

The response surfaces for 2,3-diethyl-5-methylpyrazine are shown in Figures 6 A-C and the corresponding equations in Table S1. The most noticeable difference between the treatments is the relative concentration of 2,3-diethyl-5-methylpyrazine in FJS flour which was approximately five and three times bigger than in flour from DJS and AJS respectively. Figure 6 clearly demonstrates the positive influence of time and temperature on the formation of this compound. However, in AJS and FJS flour, none of the coefficients relating to roast time or temperature had a significant impact on the response at $p < 0.05$, either linear or quadratic, although they were significant at $p < 0.1$. P-values were 0.07 and 0.09 respectively and positive, confirming the positive effect of temperature.

Direct comparison of the formation of 2,3-diethyl-5-methylpyrazine at the lowest and highest temperature ($t = 40$ min in all cases) showed that it was significantly higher in all three flours when the higher temperature was employed (Table 5). Furthermore, in DJS, four out of the six pyrazines monitored also showed a significant increase (all at $p < 0.001$) and in AJS five out of six showed a significant increase (four of these at $p < 0.001$). This is in agreement with many other studies^{10,37} that show that pyrazine formation in general is greatly influenced by temperature. Queiroz and Garcia³² evaluated roasting time and temperature for cupuaçu seeds and concluded that increased time resulted in greater pyrazine formation and increased the scores for chocolate in the sensory profile. For cupuaçu, the best roasting conditions were 150°C for 42 min. For cocoa beans, Farah et al.¹⁰ reported an increase in the concentration of pyrazines, particularly tetramethylpyrazine, when they roasted beans at temperatures close to 160°C .

Figure 6 (A, B and C) shows that the greatest relative concentration of 2,3-dimethyl-5-methylpyrazine was formed in dry, acidified and fermented flour when we

used 171 or 186, 201 and 180°C, respectively. These temperatures are higher than those milder conditions (110 - 140 °C for 20 - 50 min) reported for cocoa by Jinap et al.³⁶ or Afoakwa et al.¹³ (120-150 °C for 5-120 min).

The response surfaces for 3-methylbutanal are shown in Figures 6 D-F. They clearly demonstrate that, contrary to 2,3-diethyl-5-methylpyrazine, high time and temperature are not the most favorable roasting conditions for the formation of 3-methylbutanal. The equation in Table S1 shows that the linear temperature coefficient in AJS is significant ($p=0.03$) and negative, indicating that the lower temperatures produce a greater response. For AJS and FJS, the lowest temperatures generated the most 3-methylbutanal, but in DJS, there was an optimum around the mid-point, consistent with the data presented in Table 5. Optimum temperatures for 3-methylbutanal in DJS, AJS and FJS were 171, 165 and 154 °C, respectively, closer to those used for cocoa roasting.

The similarity of the optimum jackfruit roasting conditions, compared to cocoa, may be due to the fact that jackfruit seeds have a similar composition compared to cocoa beans, although dried jackfruit seeds have a lower lipid content (0.4% compared to dried cocoa beans which have range between 53 and 39%).^{12,13}

Whilst we have selected a few compounds as a marker of chocolate flavor, it is clear from these results that there are other factors involved, particularly those associated with the fermented product. Further work is currently being carried out to investigate more thoroughly the contribution from a wider range of volatile compounds.

Waste jackfruit seeds have been roasted to prepare a flour which has a chocolate aroma. Moisture, pH and color were similar to those of cocoa, and different aroma profiles were obtained by acidifying or fermenting the seeds prior to roasting under

different time/temperature combinations. Optimum chocolate aroma scores were achieved when either fermentation or acidification was performed prior to roasting, and fermentation produced fewer off-notes. Utilization of this local waste stream can provide a new revenue stream for local farmers and boost local economies.

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ASSOCIATED CONTENT

Table S1: Equations, coefficients, r^2 and p-value for all equations derived from the response surface methodology. Table S2 Summary of odor-active compounds found in chocolate and cocoa 1997-2017. This material is available free of charge via the Internet at <http://pubs.acs.org>.

- 536 (1) Saxena, A.; Bawa, A. S.; Raju, P. S. Jackfruit (*Artocarpus heterophyllus* Lam.). In
537 *Postharvest Biology and Technology of Tropical And Subtropical Fruits*; Yahia, E.
538 M., Ed.; Woodhead: Philadelphia, 2011; pp 275–298.
- 539 (2) Mahanta, C. L.; Kalita, D. Processing and utilization of jackfruit seeds. In
540 *Processing and Impact on Active Components in Food*; Preedy, V. R., Ed.;
541 Elsevier: London, U.K., 2015; pp 395–400.
- 542 (3) Madruga, M. S.; De Albuquerque, F. S. M.; Silva, I. R. A.; Do Amaral, D. S.;
543 Magnani, M.; Neto, V. Q. Chemical, morphological and functional properties of
544 Brazilian jackfruit (*Artocarpus heterophyllus* L.) seeds starch. *Food Chem.* **2014**,
545 *143*, 440–445.
- 546 (4) Rengsutthi, K.; Charoenrein, S. Physico-chemical properties of jackfruit seed
547 starch (*Artocarpus heterophyllus*) and its application as a thickener and stabilizer
548 in chilli sauce. *Lebensm. Wiss. Technol.* **2011**, *44*, 1309–1313.
- 549 (5) Mukprasirt, A.; Sajjaanantakul, K. Physico-chemical properties of flour and
550 starch from jackfruit seeds (*Artocarpus heterophyllus* Lam.) compared with
551 modified starches. *Int. J. Food Sci. Technol.* **2004**, *39*, 271–276.
- 552 (6) Tulyathan, V.; Tananuwong, K.; Songjinda, P.; Jaiboon, N. Some physicochemical
553 properties of jackfruit (*Artocarpus heterophyllus* Lam) seed flour and starch.
554 *ScienceAsia* **2002**, *28*, 37–41.
- 555 (7) Schnermann, P.; Schieberle, P. Evaluation of key odorants in milk chocolate and
556 cocoa mass by aroma extract dilution analysis. *J. Agric. Food Chem.* **1997**, *45*,
557 867–872.
- 558 (8) Counet, C.; Callemien, D.; Ouwerx, C.; Collin, S. Use of Gas
559 Chromatography–olfactometry to identify key odorant compounds in dark
560 chocolate. Comparison of samples before and after conching. *J. Agric. Food*
561 *Chem.* **2002**, *50*, 2385–2391.
- 562 (9) Seitz, E. W. Fermentation production of pyrazines and terpenoids for flavour
563 and fragrances. In *Bioprocess Production of Flavour, Fragrance, and Color*
564 *Ingredients*; Gabelman, A., Ed.; Wiley: New York, 1994; pp 95–134.
- 565 (10) Farah, D. M. H.; Zaibunnisa, A. H.; Misnawi. Optimization of cocoa beans
566 roasting process using response surface methodology based on concentration of
567 pyrazine and acrylamide. *Int. Food Res. J.* **2012**, *19*, 1355–1359.
- 568 (11) Tran, P. D.; Van de Walle, D.; De Clercq, N.; De Winne, A.; Kadow, D.; Lieberei,
569 R.; Messens, K.; Tran, D. N.; Dewettinck, K.; Van Durme, J. Assessing cocoa
570 aroma quality by multiple analytical approaches. *Food Res. Int.* **2015**, *77*, 657–
571 669.

- 572 (12) Gu, F.; Tan, L.; Wu, H.; Fang, Y.; Xu, F.; Chu, Z.; Wang, Q. Comparison of cocoa
573 beans from China, Indonesia and Papua New Guinea. *Foods* **2013**, *2*, 183–197.
- 574 (13) Afoakwa, E. O. Cocoa cultivation, bean composition and chocolate flavour
575 precursor formation. In *Chocolate Science and Technology*; Afoakwa, E. O., Ed.;
576 Wiley Blackwell: Oxford, U.K., 2010; pp 12–34.
- 577 (14) Parker, J. K. Thermal generation of aroma. In *Flavour Development, Analysis and*
578 *Perception in Food and Beverages*; Parker, J.K.; Elmore, J.S.; Methven, L. Eds.;
579 Woodhead Publishing: Cambridge, U.K., 2015; pp 3–30.
- 580 (15) Kongor, J. E.; Hinneh, M.; de Walle, D. Van; Afoakwa, E. O.; Boeckx, P.;
581 Dewettinck, K. Factors influencing quality variation in cocoa (*Theobroma cacao*)
582 bean flavour profile - a review. *Food Res. Int.* **2016**, *82*, 44–52.
- 583 (16) Kirchhoff, P. M.; Biehl, B.; Crone, G. Peculiarity of the accumulation of free
584 amino acids during cocoa fermentation. *Food Chem.* **1989**, *31*, 295–311.
- 585 (17) Koehler, P. E.; Odell, G. V. Factors affecting the formation of pyrazine
586 compounds in sugar-amine reactions. *J. Agric. Food Chem.* **1970**, *18*, 895–898.
- 587 (18) Ito, K.; Mori, M. Formation of pyrazines in aqueous maltose / glucose / fructose-
588 glutamine model systems upon heating at below 100°C. *Food Sci. Technol. Res.*
589 **2004**, *10*, 199–204.
- 590 (19) Ledl, F.; Schleicher, E. New aspects of the Maillard reaction in foods and in the
591 human Bbody. *Angew. Chemie-International Ed.* **1990**, *29*, 565–594.
- 592 (20) Oyekale, a. S.; Bolaji, M. B.; Olowa, O. W. The effects of climate change on cocoa
593 production and vulnerability assessment in Nigeria. *Agric. J.* **2009**, *4*, 77–85.
- 594 (21) Fairtrade Foundation. Fairtrade and Cocoa - Commodity Briefing. **2011**, No.
595 August, 1–17.
- 596 (22) FAO - Food and Agriculture. Perspectivas a plazo medio de los productos básicos
597 agrícolas. <http://www.fao.org/docrep/007/y5143s/y5143s0w.htm> (accessed Oct
598 6, 2015).
- 599 (23) AOAC Official Methods of Analysis. *Official methods of analysis of the*
600 *association of official analytical chemists*, second edition; Sidney Williams, Ed.;
601 Arlington: Virginia, 1984.
- 602 (24) AOAC Official Methods of Analysis. Official methods of analysis of the
603 association of official analytical chemists. In *Official methods of analysis of the*
604 *association of official analytical chemists*; Horwitz, D. W., Dr. George W. Latimer,
605 J., Eds.; 2010; p 6.
- 606 (25) Cochran, W. G.; Cox, G. M. *Experimental Designs*, 2nd ed.; Cochran, W. G., Cox,
607 G. M., Eds.; John Wiley and Sons Inc: New York, 1950.

- 608 (26) Dimick, P. S.; Hoskin, J. C. The chemistry of flavour development in chocolate. In
609 *Industrial chocolate manufacture and use*; Beckett, S. T., Ed.; Oxford, U.K., 1999;
610 pp 137–387.
- 611 (27) Beckett, S. T. *Industrial chocolate manufacture and use*, 3rd ed.; Beckett, S. T.,
612 Ed.; Blackwell Science, Oxford, U.K., 1999.
- 613 (28) Jinap, S.; Dimick, P. S. Acidic characteristics of fermented and dried cocoa beans
614 from different countries of origin. *J. Food Sci.* **1990**, *55*, 547.
- 615 (29) Heemskerk, R. F. M. Cleaning, roasting and winnowing. In *Industrial chocolate*
616 *manufacture and use*; Beckett, S. T., Ed.; Blackwell Science: Oxford, U.K., 1999;
617 pp 78–100.
- 618 (30) Rodriguez-Campos, J.; Escalona-Buendía, H. B.; Orozco-Avila, I.; Lugo-Cervantes,
619 E.; Jaramillo-Flores, M. E. Dynamics of volatile and non-volatile compounds in
620 cocoa (*Theobroma cacao* L.) during fermentation and drying processes using
621 principal components analysis. *Food Res. Int.* **2011**, *44*, 250–258.
- 622 (31) Yousif, A. K.; Alghzawi, H. M. Processing and characterization of carob powder.
623 *Food Chem.* **2000**, *69*, 283–287.
- 624 (32) Queiroz, M. B.; Garcia, N. H. P. Evaluation to sensory profile to roast cupuassu
625 and cocoa using QDA. **2000**, 249–266.
- 626 (33) Cohen, K. D. O.; Jackix, M. D. N. H. Estudo do liquor de cupuaçu. *Ciência e*
627 *Tecnol. Aliment.* **2005**, *25*, 182–190.
- 628 (34) Sacchetti, G.; Ioannone, F.; De Gregorio, M.; Di Mattia, C.; Serafini, M.;
629 Mastrocola, D. Non enzymatic browning during cocoa roasting as affected by
630 processing time and temperature. *J. Food Eng.* **2016**, *169*, 44–52.
- 631 (35) Hashim, P.; Selamat, J.; Syed Muhammad, S. K.; Ali, A. Changes in free amino
632 acid, peptide-N, sugar and pyrazine concentration during cocoa fermentation. *J.*
633 *Sci. Food Agric.* **1998**, *78*, 535–542.
- 634 (36) Jinap, S.; Wan Rosli, W. I.; Russly, a. R.; Nordin, L. M. Effect of roasting time and
635 temperature on volatile component profiles during nib roasting of cocoa beans
636 (*Theobroma cacao*). *J. Sci. Food Agric.* **1998**, *77*, 441–448.
- 637 (37) Owusu, M.; Petersen, M.A.; Heimdal, H. Effect of fermentation method, roasting
638 and conching conditions on the aroma volatiles of dark chocolate. *J. Food*
639 *Process. Pres.* **2012**, *36*, 446–456.
- 640 (38) Frauendorfer, F.; Schieberle, P. Identification of the key aroma compounds in
641 cocoa powder based on molecular sensory correlations. *J. Agric. Food Chem.*
642 **2006**, *54*, 5521–529.
643

644 FIGURE CAPTIONS

645 Figure 1A. Summary of jackfruit seed processing. DJS, AJS and FJS are dried, acidified
646 and fermented jackfruit seeds respectively.

647 Figure 1B. Central composite design using two factors each at 5 levels; DJS, AJS and FJS
648 are dried, acidified and fermented jackfruit seeds respectively.

649 Figure 2. Experimental design used for sensory ranking test where T= number of
650 samples; k= number of samples in each ranking test; r= number of times each sample
651 was shown within each block; B= number of panelists in each block; L= number of
652 times the samples were shown together; E = dependability of the analysis; Z= number
653 times the block was repeated.²⁵

654 Figure 3. Variables followed during the processing of the seeds prior to roasting: A = pH
655 during fermentation process; B = pH during acidification process; C = total titratable
656 acidity (g/100g) during fermentation process and D = temperature (°C) during
657 fermentation process; close = anaerobic 6-7 days; open = aerobic 7-8 days.

658 Figure 4. Response surfaces for roasted jackfruit seeds. A, B and C = total sensory
659 chocolate aroma (SCA) ranking score for flour from DJS, AJS and FJS respectively.

660 Figure 5. Representation of aroma attributes used freely by the panelists to describe
661 the roasted flours from fermented, dried and acidified jackfruit seed

662 Figure 6. Response surfaces for roasted jackfruit seeds. A, B and C = 2,3-diethyl-5-
663 methylprazine for flour from DJS, AJS and FJS respectively. D, E and F = 3-
664 methylbutanal from DJS, AJS and FJS respectively.

Table 1. Results from preliminary ranking experiment using different pre-exposure conditions of the roasted flours prior to ranking

coded values		roasting conditions		total ranking score for sensory chocolate aroma ^a
x	y	time (s)	temperature (°C)	
1	-1	120	25	13 a
-1	-1	30	25	15 a
-1	1	30	48	16 a
1	1	120	48	20 a
0	0	60	36.5	22 a
0	0	60	36.5	21 a
0	0	60	36.5	19 a

T=7; k=3; r=3; b=21; L=1; E=78 where T = number of samples; k = number of samples in each ranking test; r = number of times each sample was shown within each block; B= number of panelists in each block; L= number of times the samples were shown together; E = dependability of the analysis; Z= number times the block was repeated²⁴.

^avalues with the same letter are not significantly different at $p < 0.05$

Table 2. Mean \pm standard error (n=3) pH, water activity, moisture L* and chroma* of the roasted jackfruit seed flours showing mean values.

x (time)	y (temp)	pH	a _w	moisture %	L*	chroma
Flour from dried jackfruit seeds (DJS)						
0	1.41	5.4 \pm 0.04 ^a	0.33 \pm 0.02 ^d	1.0 \pm 0.3 ^d	53 \pm 3 ^e	33.6 \pm 0.2 ^a
1	1	5.3 \pm 0.01 ^d	0.32 \pm 0.02 ^d	1.2 \pm 0.4 ^d	55 \pm 1 ^{de}	33.2 \pm 0.2 ^{ab}
-1	1	5.3 \pm 0.02 ^{bcd}	0.32 \pm 0.01 ^d	1.3 \pm 0.3 ^d	58 \pm 3 ^{cde}	32.1 \pm 0.6 ^{bc}
-1.41	0	5.3 \pm 0.02 ^d	0.37 \pm 0.01 ^c	3.7 \pm 0.8 ^b	59 \pm 3 ^{bcd}	32.1 \pm 0.2 ^{bc}
1.41	0	5.4 \pm 0.02 ^b	0.31 \pm 0.01 ^d	2.0 \pm 0.2 ^{cd}	59 \pm 0.4 ^{bcd}	33.3 \pm 0.6 ^{ab}
0	0	5.2 \pm 0.01 ^e	0.38 \pm 0.01 ^c	3.4 \pm 0.6 ^{bc}	60 \pm 2 ^{abcde}	32.9 \pm 0.4 ^{ab}
0	0	5.3 \pm 0.01 ^d	0.42 \pm 0.03 ^{abc}	2.9 \pm 0.5 ^{bc}	60 \pm 3 ^{abcd}	33.2 \pm 0.3 ^{ab}
0	0	5.2 \pm 0.01 ^e	0.43 \pm 0.01 ^a	3.2 \pm 0.3 ^{bc}	60 \pm 2 ^{abcde}	32.5 \pm 0.5 ^{abc}
1	-1	5.2 \pm 0.01 ^e	0.44 \pm 0.01 ^a	5.8 \pm 0.9 ^a	64 \pm 2 ^{abc}	31.6 \pm 0.5 ^c
-1	-1	5.4 \pm 0.00 ^b	0.40 \pm 0.02 ^{abc}	5.8 \pm 0.8 ^a	66.0 \pm 1.6 ^a	31.3 \pm 0.6 ^c
0	-1.41	5.3 \pm 0.01 ^{bc}	0.40 \pm 0.01 ^{bc}	6.4 \pm 0.2 ^a	65.1 \pm 1.5 ^{ab}	31.2 \pm 0.5 ^c
Flour from acidified jackfruit seeds (AJS)						
0	1.41	5.6 \pm 0.01 ^a	0.5 \pm 0.01 ^{abc}	0.5 \pm 0.4 ^d	61.7 \pm 0.9 ^{cd}	32.0 \pm 0.8 ^a
1	1	5.6 \pm 0.01 ^a	0.50 \pm 0.02 ^a	0.9 \pm 0.9 ^{cd}	60.5 \pm 1.6 ^d	31.5 \pm 0.8 ^a
-1	1	5.2 \pm 0.01 ^f	0.47 \pm 0.01 ^c	1.6 \pm 0.2 ^{bcd}	65.3 \pm 0.9 ^{abcd}	30.2 \pm 0.2 ^{ab}
1.41	0	5.3 \pm 0.01 ^c	0.47 \pm 0.01 ^{bc}	1.9 \pm 0.7 ^{abcd}	66.1 \pm 0.8 ^{abc}	31.1 \pm 0.2 ^a
1.41	0	5.2 \pm 0.01 ^{de}	0.48 \pm 0.01 ^{abc}	2.4 \pm 0.6 ^{abc}	64.7 \pm 2.2 ^{abcd}	30.5 \pm 0.7 ^{ab}
0	0	5.3 \pm 0.01 ^{cd}	0.50 \pm 0.01 ^{ab}	1.9 \pm 0.7 ^{bcd}	66.1 \pm 1.4 ^{abc}	30.2 \pm 0.5 ^{ab}
0	0	5.2 \pm 0.01 ^{ef}	0.47 \pm 0.01 ^{bc}	2.0 \pm 0.5 ^{bcd}	65.3 \pm 0.4 ^{abcd}	30.8 \pm 0.5 ^{ab}
0	0	4.9 \pm 0.02 ^h	0.47 \pm 0.01 ^{bc}	1.4 \pm 0.7 ^{bcd}	64.3 \pm 3.0 ^{abcd}	31.1 \pm 1.0 ^a
1	-1	5.0 \pm 0.02 ^g	0.46 \pm 0.01 ^c	2.6 \pm 0.8 ^{ab}	63.2 \pm 2.9 ^{bcd}	31.2 \pm 0.8 ^a
-1	-1	5.2 \pm 0.03 ^{ef}	0.48 \pm 0.01 ^{abc}	2.9 \pm 0.2 ^{ab}	67.3 \pm 1.9 ^{ab}	30.6 \pm 0.8 ^{ab}
0	-1.41	5.3 \pm 0.01 ^b	0.48 \pm 0.01 ^{abc}	3.8 \pm 0.2 ^a	69.3 \pm 0.8 ^a	29.1 \pm 0.2 ^b
Flour from fermented jackfruit seeds (FJS)						
0	1.41	5.1 \pm 0.00 ^a	0.34 \pm 0.01 ^e	2.3 \pm 0.5 ^f	49.4 \pm 1.9 ^{ab}	27.6 \pm 0.5 ^{ab}
1	1	5.0 \pm 0.01 ^b	0.42 \pm 0.01 ^{ab}	2.3 \pm 0.1 ^f	50.1 \pm 1.8 ^{ab}	27.8 \pm 0.4 ^{ab}
-1	1	4.8 \pm 0.01 ^c	0.39 \pm 0.01 ^{cd}	2.5 \pm 0.2 ^f	53.5 \pm 1.3 ^a	28.1 \pm 0.3 ^{ab}
-1.41	0	4.7 \pm 0.01 ^g	0.38 \pm 0.01 ^d	4.1 \pm 0.5 ^{bcd}	52.3 \pm 3.5 ^a	28.9 \pm 0.7 ^a
1.41	0	4.8 \pm 0.01 ^d	0.43 \pm 0.01 ^a	3.6 \pm 0.1 ^{cde}	53.7 \pm 0.6 ^a	28.8 \pm 0.4 ^a
0	0	4.8 \pm 0.02 ^{ef}	0.39 \pm 0.01 ^{cd}	3.3 \pm 0.3 ^{def}	52.8 \pm 1.9 ^a	27.7 \pm 0.5 ^{ab}
0	0	4.7 \pm 0.00 ^f	0.40 \pm 0.01 ^{bc}	3.0 \pm 0.4 ^{ef}	44.5 \pm 3.4 ^b	26.8 \pm 0.8 ^b
0	0	4.8 \pm 0.01 ^d	0.37 \pm 0.01 ^d	3.9 \pm 0.5 ^{cde}	49.5 \pm 2.1 ^{ab}	28.4 \pm 0.4 ^a
1	-1	4.7 \pm 0.01 ^g	0.41 \pm 0.01 ^{ab}	4.5 \pm 0.1 ^{bc}	51.5 \pm 1.1 ^a	27.7 \pm 0.3 ^{ab}
-1	-1	4.5 \pm 0.02 ^h	0.41 \pm 0.01 ^{ab}	5.7 \pm 0.2 ^a	51.3 \pm 2.9 ^a	27.9 \pm 0.4 ^{ab}
0	-1.41	4.8 \pm 0.01 ^{de}	0.38 \pm 0.01 ^{cd}	5.1 \pm 0.4 ^{ab}	49.0 \pm 2.9 ^{ab}	27.9 \pm 0.4 ^{ab}

Within each column for each treatment, values with the same letter are not significantly different from each other ($p \leq 0.05$) using the Tukey test.

Table 3. Proximate composition (% \pm standard error) of jackfruit flours roasted under the best conditions.

flour ^a	moisture	lipids	proteins	ash	fiber	
					insoluble	soluble
DJS	5.88 \pm 0.9 a ^b	0.40 \pm 0.05 a	11.20 \pm 0.7 b	2.90 \pm 0.02 b	10.34 \pm 0.05 b	3.88 \pm 0.010 a
AJS	1.38 \pm 0.7 b	0.30 \pm 0.05 b	11.16 \pm 0.5 b	2.44 \pm 0.12 c	9.29 \pm 0.01 c	2.68 \pm 0.003 b
FJS	5.10 \pm 0.4 a	0.50 \pm 0.03 a	14.82 \pm 0.5 a	4.70 \pm 0.09 a	18.9 \pm 0.8 a	3.34 \pm 0.002 a

^aDJS = dried jackfruit seeds (47min at 171 °C); AJS = acidified jackfruit seeds (40 min at 180 °C); FJS =fermented jackfruit seeds (40 min at 150 °C)

^bMean (n=3), within each column, values with the same letter are not significantly different from each other ($p \leq 0.05$) using the Tukey test.

Table 4. Total sensory chocolate aroma (SCA) ranking score for flour from DJS, AJS and FJS.

	coded values		total ranking scores for sensory chocolate aroma ^a		
	x (time)	y (temp)	DJS	AJS	FJS
incomplete block					
1	1	-1	66 a ^b	67 ab	68 ab
2	-1	-1	52 a	58 ab	66 ab
3	-1	1	58 a	63 ab	60 ab
4	1	1	66 a	51 ab	51 bc
5	0	1.41	51 a	42 c	44 c
6	0	-1.41	57 a	54 bc	72 a
7	1.41	0	62 a	69 a	61 ab
8	-1.41	0	56 a	66 ab	55 bc
blend	0	0	62 a	70 a	63 ab
complete block ^c					
9	0	0	64 k	68 k	71 k
10	0	0	62 k	68 k	65 k
11	0	0	60 k	74 k	53 k

^aDJS = dried jackfruit seeds; AJS = acidified jackfruit seeds; FJS - fermented jackfruit seeds.

^bWithin each column, means followed by the same letters are not significantly different ($p \leq 0.05$).

^cValues are transformed for comparison with incomplete block.

Table 5. Approximate relative concentrations of selected volatiles in roasted jackfruit seed flours

LRI ^a	compound ID ^b	roasting conditions											significance ^c		
													T	t	Txt
DRIED JACKFRUIT (DJS)															
	roasting temp	150 °C	156 °C	156 °C	171 °C	171 °C	171 °C	171 °C	171 °C	186 °C	186 °C	192 °C			
	roasting time	40 min	35 min	45 min	33 min	40 min	40 min	40 min	47 min	35 min	45 min	40 min			
657	3-methylbutanal	6.0d ^d	7.1 bcd	8.1 abc	8.8 ab	8.1 abc	9.7 a	6.5 cd	5.4 d	7.2 bcd	5.6 d	3.4 e	***	*	ns
827	2-methylpyrazine	4.3 fg	3.3 g	4.6 fg	6.6 efg	15 d	12 de	11 def	124 a	25 c	33 b	32 b	***	***	ns
916	2,5/6-dimethylpyrazine	39 e	54 e	66 de	101 cd	121 bc	124 bc	96 cd	123 bc	167 a	185 a	155 ab	***	ns	ns
922	2,3-dimethylpyrazine	12 g	11 g	72 b	19 fg	30 def	34 d	21 efg	33 de	49 c	63 b	110 a	***	***	***
1008	2,3,5-trimethylpyrazine	25 abc	23 abc	11 c	19 bc	25 abc	26 abc	19 bc	44 ab	42 a	36 ab	35 ab	*	ns	ns
1058	phenylacetaldehyde	11.2a	8.9ab	9.7a	8.1ab	6.6abc	7.8ab	10.6a	6.8abc	6.8abc	1.9c	3.4bc	***	ns	ns
1091	2,3,5,6-tetramethyl- pyrazine	110 cde	92 cde	68 e	69 e	88 e	139 bcd	110 cde	91 de	180 ab	210 a	140 bc	***	ns	ns
1157	2,3-diethyl-5-methyl- pyrazine	1.5 fg	1.3 g	1.9 ef	1.9 ef	2.6 cd	3.2 b	2.2 de	4.3 a	3.9 a	4.3 a	3 bc	***	***	ns
1263	2-phenylethyl acetate	0.1 ab	0.1 ab	0.08 bc	0.09 bc	0.08 c	0.07 c	0.08 bc	0.12 a	0.13 a	0.12 a	0.08 c	**	***	ns
ACIDIFIED JACKFRUIT (AJS)															
	roasting temp	159 °C	165 °C	165 °C	180 °C	180 °C	180 °C	180 °C	180 °C	195 °C	195 °C	201 °C			

	roasting time	40 min	35 min	45 min	33 min	40 min	40 min	40 min	47 min	35 min	45 min	40 min			
657	3-methylbutanal	11.2 bc	13.2 a	12.2 ab	10.3 c	10.1 c	4.4 ef	7.5 d	7.8 d	5.2 e	4 f	2.3 g	***	*	ns
827	2-methylpyrazine	2.7 d	3.5 d	11.6 c	1.2 d	1.6 d	28 a	21 b	11 c	2.6 d	25 ab	29 a	**	**	ns
916	2,5/6-dimethylpyrazine	66 d	74 d	110 c	110 c	120 bc	146 a	137 ab	86 d	113 c	140 ab	123 bc	***	***	ns
922	2,3-dimethylpyrazine	37 g	126 a	58 f	59 f	68 ef	97 c	79 d	8.7 h	73 de	110 b	110 bc	***	***	***
1008	2,3,5-trimethylpyrazine	52 abc	65 a	28 cd	30 bcd	35 abcd	44 abc	43 abc	18 d	45 abc	59 abc	65 ab	ns	*	*
1058	phenylacetaldehyde	10.7bc	11.8ab	10.8b	9.7bcd	7.4cd	14.8a	7.1d	3.3e	3.4e	2.7e	0.9e	ns	**	ns
1091	2,3,5,6-tetramethylpyrazine	28 cd	67 bc	38 bcd	44 bcd	59 bcd	57 bcd	79 bc	12 d	88 b	160 a	150 a	***	ns	**
1157	2,3-diethyl-5-methylpyrazine	1.2 f	1.1 f	1.8 ef	2.1 de	2.8 d	4.6 c	4.2 c	1.8 ef	4.9 bc	7.8 a	5.4 b	***	***	**
1263	2-phenylethyl acetate	0.13 d	0.14 d	0.16cd	0.14 d	0.17bcd	0.15d	0.19abc	0.07e	0.20ab	0.21a	0.19abc	***	***	ns
FERMENTED JACKFRUIT SEEDS (FJS)															
	roasting temp	150 °C	154 °C	154 °C	165 °C	165 °C	165 °C	165 °C	165 °C	176 °C	176 °C	180 °C			
	roasting time	40 min	35 min	45 min	33 min	40 min	40 min	40 min	47 min	35 min	45 min	40 min			
657	3-methylbutanal	7 abcde	11 a	9.8 ab	9 abc	8.4 abcd	5.1 def	6.8 bcde	4.3 ef	5.9 cdef	2.3 f	2.8 f	***	*	ns
827	2-methylpyrazine	100 abc	86 cd	123 ab	86 bcd	100 abc	53 d	130 a	129 a	123 a	113 abc	110 abc	ns	ns	ns
916	2,5/6-dimethylpyrazine	375 ab	280 bc	450 a	250 bc	290 bc	234 c	312 bc	266 bc	278 bc	215 c	240 c	*	ns	*
922	2,3-dimethylpyrazine	487 ab	410 bc	600 a	420 bc	400 bc	421 bc	513 ab	510 ab	511 ab	402 bc	320 c	*	ns	**
1008	2,3,5-trimethylpyrazine	560 abc	480 bc	690 a	380 bcd	370 cd	110 e	130 e	98 e	210 de	130 e	270 de	***	ns	ns

1058	phenylacetaldehyde	20b	27a	20b	15bc	11cde	10cde	13c	12cd	10cde	6.9de	6.0e	***	ns	ns
1091	2,3,5,6-tetramethyl-pyrazine	4330 ab	3840 bc	5300 a	4380 ab	4070 b	3840 bc	5320 a	4190 b	4200 b	2700 d	2830 cd	**	ns	**
1157	2,3-diethyl-5-methyl-pyrazine	7.5 f	11 ef	14 bcd	12 de	16 abc	13 cde	18 a	16 ab	16 ab	19 a	15abc	***	*	ns
1263	2-phenylethyl acetate	7.3 c	12 a	11 ab	7.6 c	7 cd	6.6 cd	9.5 b	10.1 b	7.6 c	6.3 cd	5.5 b	***	*	ns

^a Linear retention index on RTX5MS column (30m), calculated from a linear equation between each pair of straight chain alkanes C₆–C₃₀.

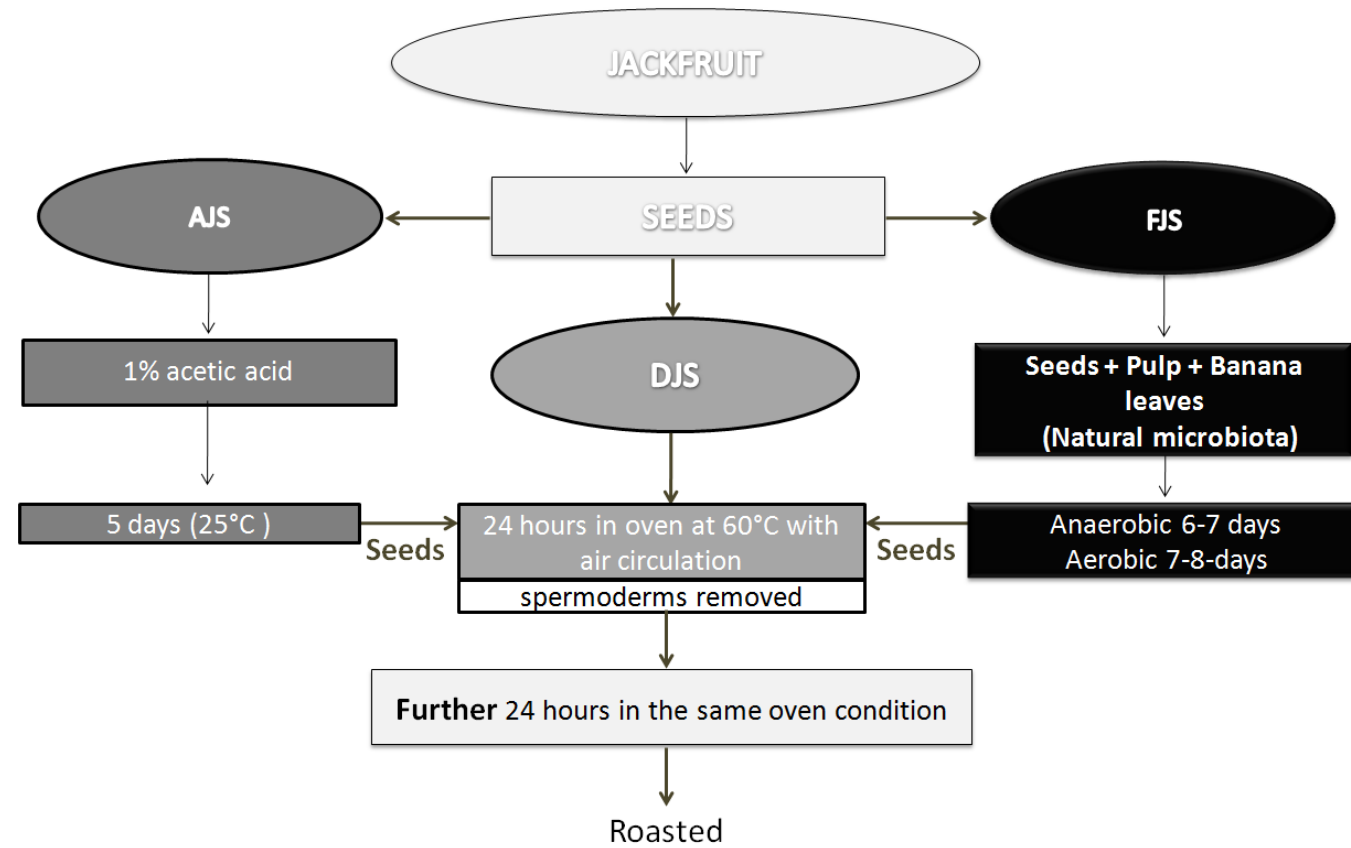
^b Identity: identity of all compounds confirmed by comparison of mass spectrum and LRI with that of the authentic standard run under similar conditions.

^cS: Significance of differences between samples within one pre-treatment (AJS, FJS or DJS) - probability, obtained from 2-way ANOVA, that there is a difference between means; ns = no significant difference between means (p>0.05); * significant at the 5% level; ** significant at the 1% level; *** significant at the 0.1% level,

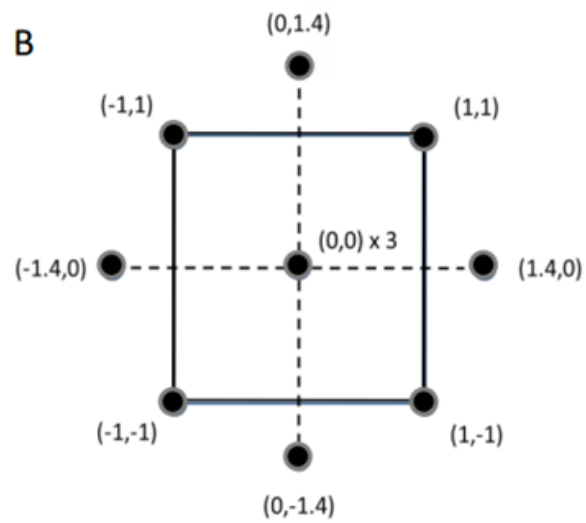
with respect to; T = roasting temperature, t = roasting time, T×t interaction between roasting time and temperature.

^d Mean (n=3) relative concentration (μg/kg) = peak area of compound × concentration of internal standard (ISTD) / peak area of ISTD, nd = not detected. Within each row, cells containing the same letter are not significantly different from each other at p<0.05.

A



B



Factors		Levels				
		-1.41	-1	0	1	1.41
Time (min), x		33	35	40	45	47
	DJS	150	156	171	186	192
	AJS	159	165	180	195	201
Temperature (°C), y	FJS	150	154	165	176	180

Figure 1A.

Figure 1B.

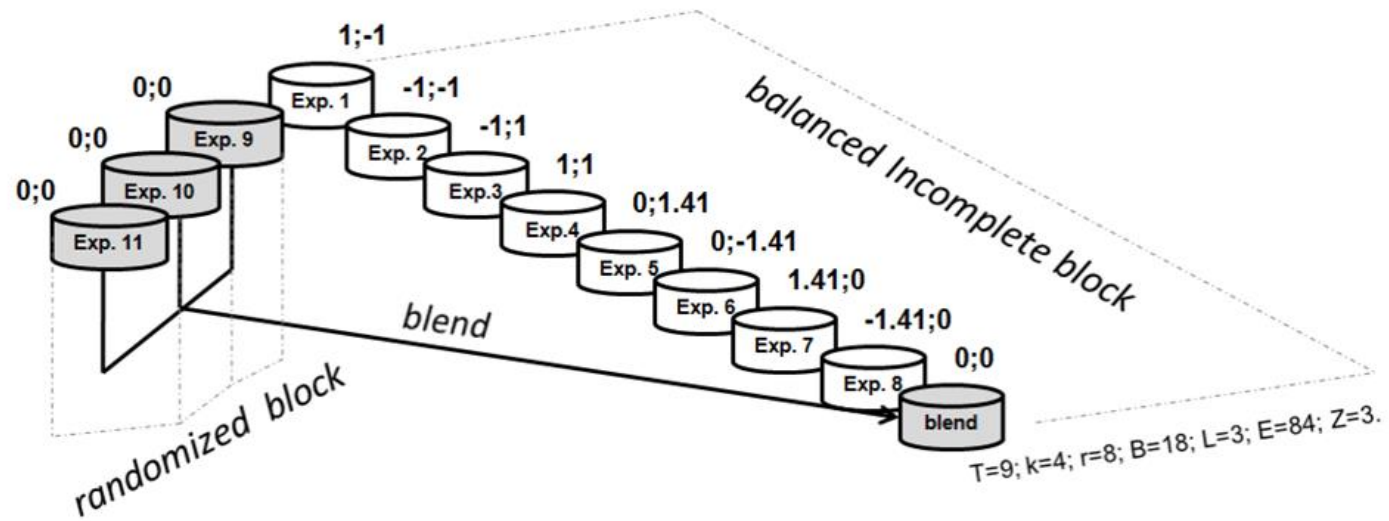


Figure 2

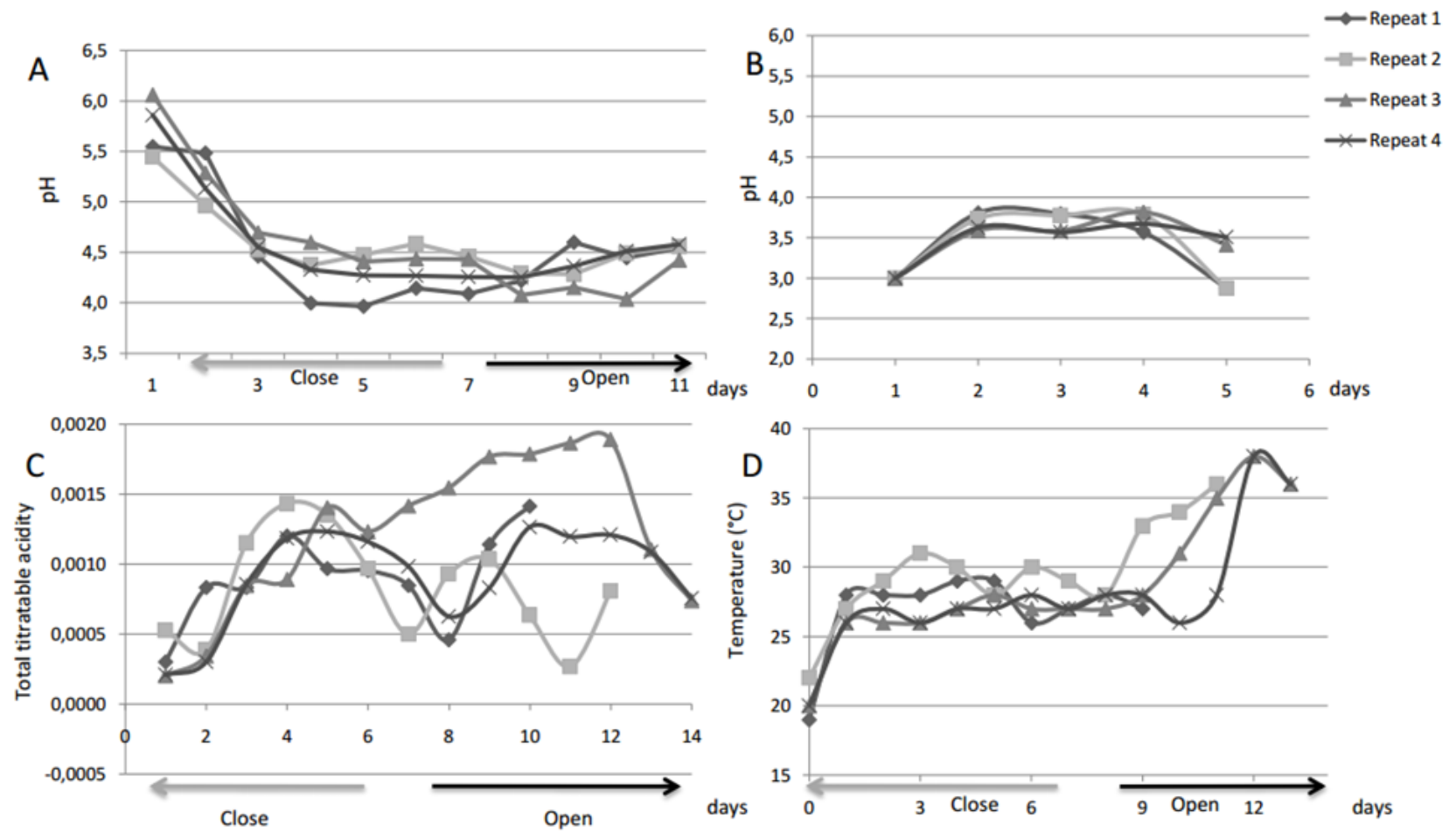
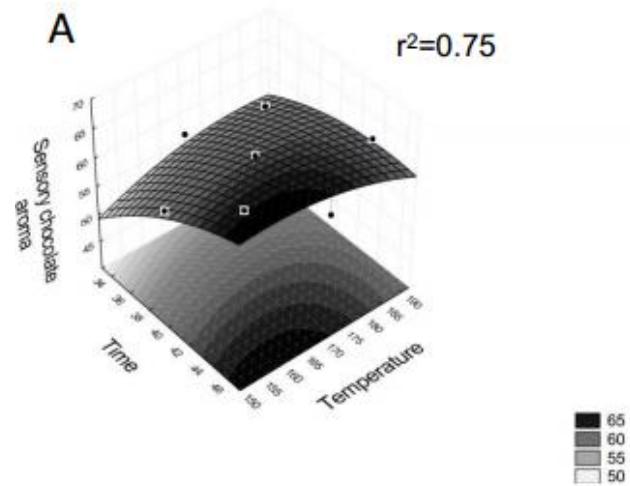
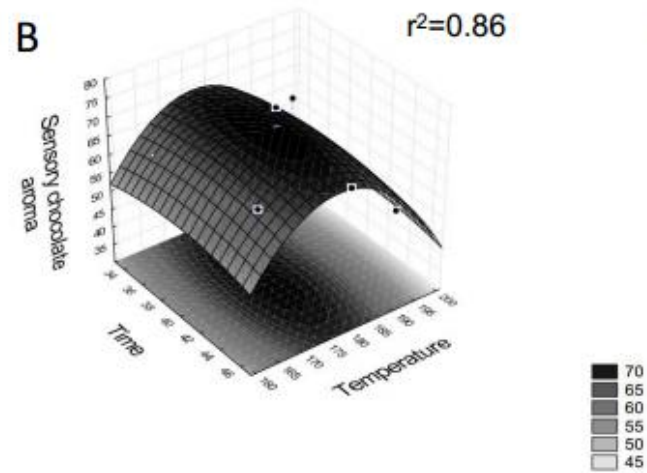


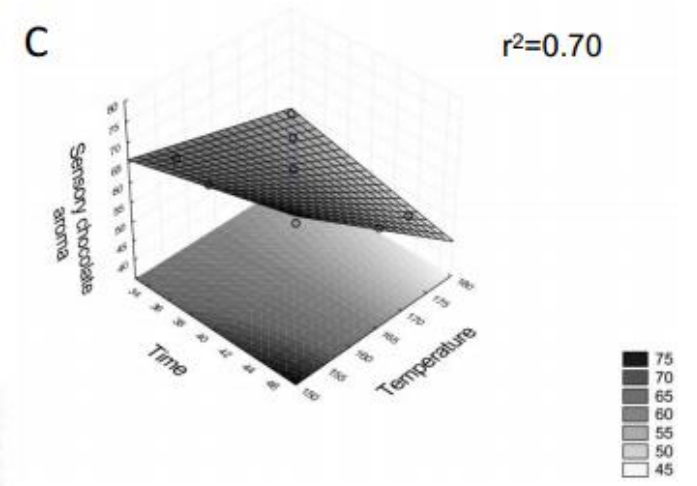
Figure 3



$$SCA_{DJS} = -324.84 + 7.807x + 2.514y - 0.0452x^2 - 0.005y^2 - 0.02xy$$



$$SCA_{AJS} = -2017.58 + 15.835x + 19.9425y - 0.0628839x^2 - 0.04942y^2 - 0.06xy$$



$$SCA_{FJS} = -621.7 + 13.46x + 5.761y - 0.0608x^2 - 0.0134y^2 - 0.05188xy$$

Figure 4

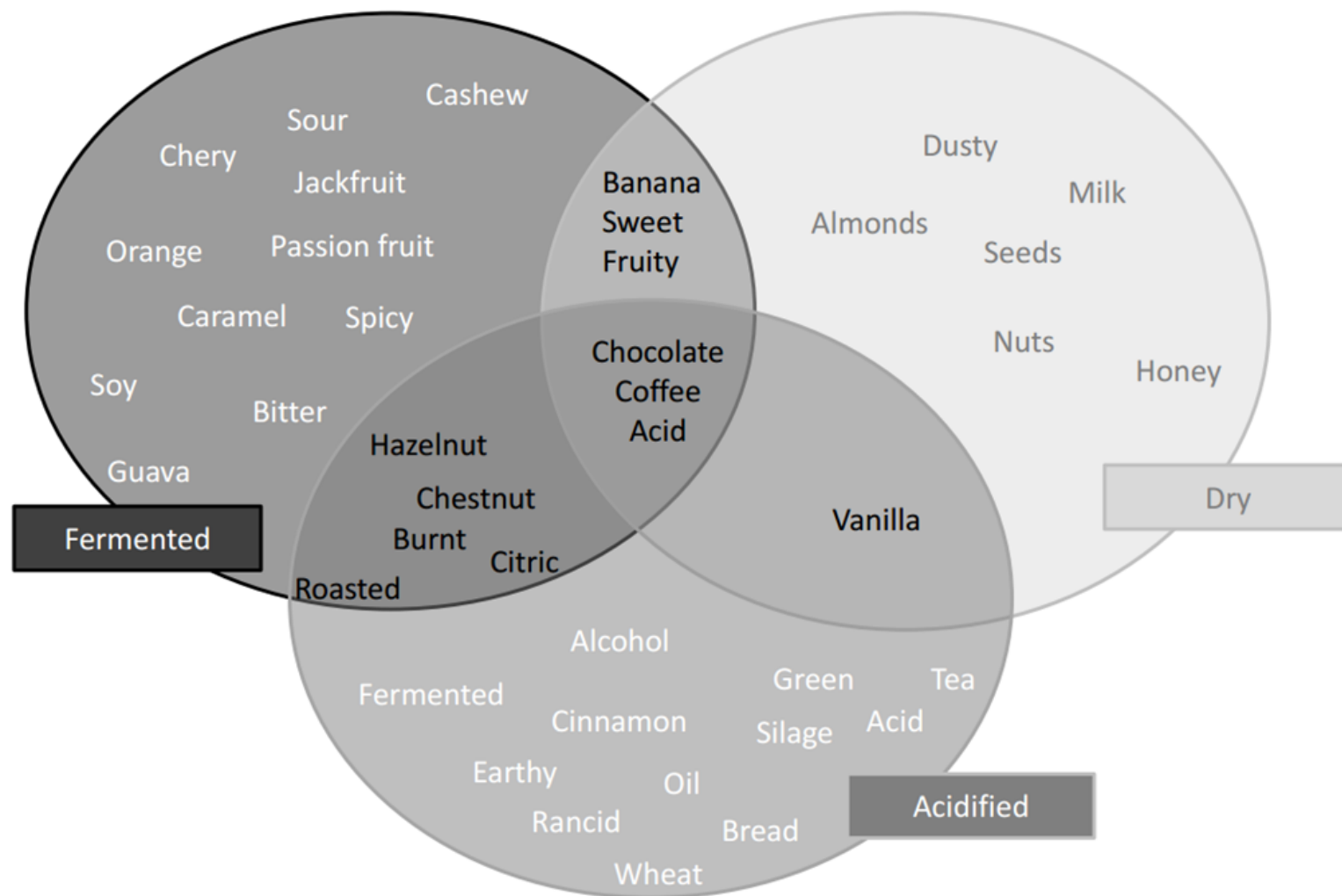


Figure 5

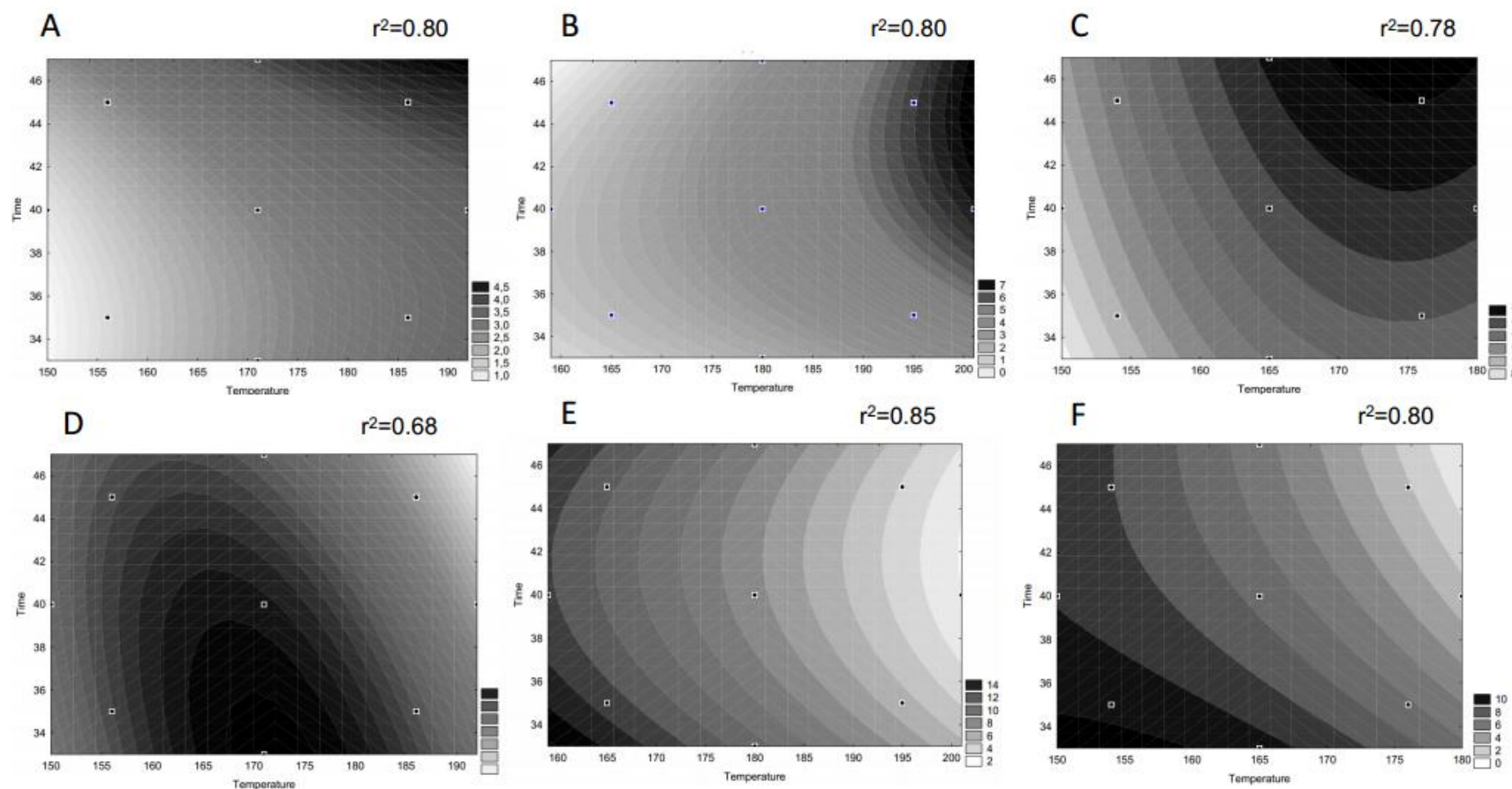


Figure 6

TOC GRAPHIC

